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14. ABSTRACT

The ingestion of food antigens plays an essential role in the development of eosinophilic esophagitis (EE) as total removal of dietary antigens by using an amino acid based oral formula improves clinical symptoms and esophageal histology in 98% of patients with EE within a month. EE is thought to be mediated by both IgE and non-IgE mediated food allergy. In this study we are particularly interested in identifying genes in EE linked to a significant complication of EE namely esophageal stricture formation. This study focuses on increasing our understanding of two genes (TGF-b and acidic chitinase) associated with remodeling and stricture formation in the esophagus in EE. The importance of TGF-b and acidic chitinase to the development of egg induced remodeling of the esophagus is being studied in a mouse model of egg induced EE in which either TGF-b signaling or acidic chitinase activity is neutralized. Our studies demonstrate that targeting Smad3 or chitinase both partially reduced egg induced esophageal fibrosis associated with EE. The combination of targeting Smad3 and chitinase may be more effective than targeting each pathway alone.

15. SUBJECT TERMS

Eosinophils, smad-3, chitinase, remodeling, food allergy

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Introduction:
The ingestion of food antigens plays an essential role in the development of eosinophilic esophagitis (EE) as total removal of dietary antigens by using an amino acid based oral formula improves clinical symptoms and esophageal histology in 98% of patients with EE within a month. EE is thought to be mediated by both IgE and non-IgE mediated food allergy. In this study we are particularly interested in identifying genes in EE linked to a significant complication of EE namely esophageal stricture formation. This study focuses on increasing our understanding of two genes namely a) TGF-b (transforming growth factor-b), and b) acidic chitinase, to determine their role in remodeling and stricture formation in the esophagus in EE. The importance of TGF-b and acidic chitinase to the development of egg induced remodeling of the esophagus is being studied in a mouse model in which either TGF-b signaling is inhibited or acidic chitinase activity is neutralized. TGF-b will be inactivated in studies of Smad3 deficient mice (essential for TGF-b signaling) and chitinase will be inactivated in studies of mice administered allosamidin a pharmacologic inhibitor of chitinase.

Body:
This proposal outlines 4 tasks to be completed during the three year proposal. We have completed Task 1 and worked on Task 2 and Task 3 in year 2 of this proposal as planned in our original proposal.

1. Task 1: Breeding of Smad3 deficient mice (month 1-3)
We have completed task 1 the breeding of Smad 3 deficient mice and used them in experiments proposed for task 2.

2. Task 2: Mouse model of egg induced EE (WT vs Smad3 deficient) (month 4-16)
We have completed:
Task 2 (a) Exposure of WT and Smad3 deficient mice to OVA allergen
Task 2 (b) Quantitating fibrosis
Task 2 (c) Quantitating basal zone hyperplasia
Task 2 (e) Quantitating eosinophils
Task 2 (f) Quantitating TGF-b+ cells

We are currently quantitating:
Task 2 (d) Quantitating blood vessels
Task 2 (g) Quantitating pSmad+ cells

The results of Task 2 are presented in the Key Research Accomplishments

3. Task 3: Mouse model of egg induced EE (WT vs WT + allasomidin a pharmacologic inhibitor of chitinase) (month 16-28)
We have completed:
Task 3 (a) Exposure of WT and WT + allasomidin to OVA allergen
Task 3 (b) Quantitating fibrosis
Task 2 (e) Quantitating eosinophils

We are currently quantitating:
Task 3 (c) Quantitating basal zone hyperplasia
Task 3 (d) Quantitating blood vessels
Task 3 (f) Quantitating TGF-b+ cells
Task 3 (g) Quantitating pSmad+ cells

The results of Task 3 are presented in the Key Research Accomplishments

Key Research Accomplishments:
- Smad-3 deficient mice have significantly less esophageal fibrosis in a mouse model of egg induced EE.

The first aim of the study was to determine whether Smad-3 deficient mice have reduced remodeling of the esophagus in a mouse model of egg (i.e. OVA) induced eosinophilic esophagitis. The studies we performed confirmed this hypothesis as Smad3 KO mice challenged intragastrically with OVA had significantly less esophageal fibrosis (assessed by quantitating the area of esophageal trichrome staining which stains collagen) compared to WT mice challenged intragastrically with OVA ($p<0.001$)(**Fig 1**). As Smad-3 mediates TGF-b signaling these studies suggest that the TGF-

b/TGF-b receptor/Smad-3 pathway is activated in inducing esophageal fibrosis. The cellular source of TGF-b in EE are predominantly esophageal eosinophils, whereas the cell that responds to TGF-b released by eosinophils are esophageal fibroblasts which express TGF-b receptors which activate intracellular Smad-3 signaling pathways to promote fibrosis. Thus, as anticipated the number of esophageal eosinophils (**Fig 2**) and esophageal cells expressing TGF-b (**Fig 3**) was not significantly different in WT and Smad-3 deficient mice challenged intragastrically with OVA. The key difference between WT and Smad3 deficient mice was the inability of Smad3 deficient mouse esophageal fibroblasts to respond to TGF-b by activating the Smad3 pathway. These results suggest that targeting Smad3 with an oral small molecule inhibitor may be a novel way to inhibit esophageal fibrosis and esophageal stricture formation which is a major complication of human EE.

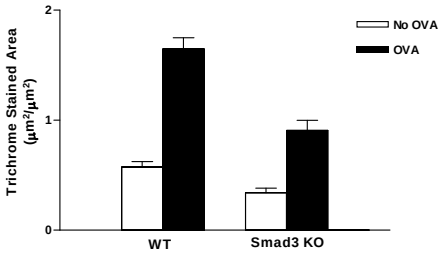


Fig 1: Trichrome stain

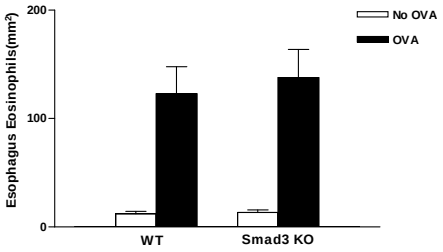


Fig 2: Eosinophils

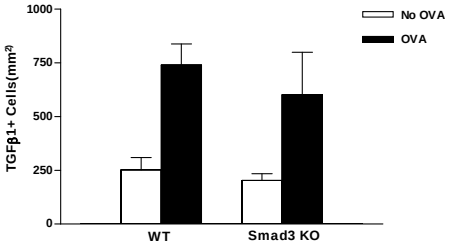


Fig 3: TGF-b positive cells

- <u>Smad-3 deficient mice have a trend for reduced basal zone hyperplasia in a mouse model of egg induced EE.</u>

Although there was a trend for OVA challenged smad-3 deficient mice to have reduced basal zone hyperplasia compared to WT mice challenged with OVA, this was not statistically significant (**Fig 4**) (p=NS). Basal zone hyperplasia may be partially mediated by TGF-b, as well as by additional cytokines and mediators other than TGF-b. This may explain why smad3 deficient mice only have a partial reduction in basal zone hyperplasia.

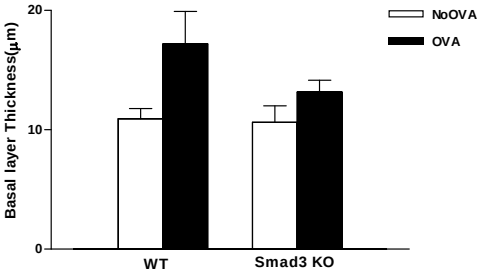
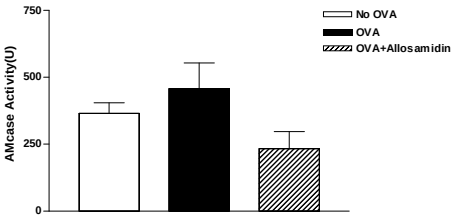
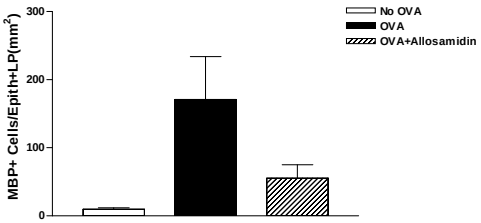


Fig 4: Basal layer thickness

- <u>Targeting chitinase with allosamidin reduces eosinophilic inflammation and esophageal fibrosis in a mouse model of egg induced EE.</u>

The second aim of this study investigated whether pharmacologic inhibition of chitinase (using allosamedin) inhibited esophageal eosinophilic inflammation and remodeling in a mouse model of OVA (egg) induced EE. Allosamedin significantly reduced AMcase activity in the esophagus of WT mice challenged with OVA and treated with allosamedin (**Fig 5**) ($P<0.01$). WT mice administered allosamedin had a significant reduction in total Major Basic Protein+ eosinophils (detected by immunohistochemistry) in esophageal epithelium and lamina propria (**Fig 6)**($p<0.001$). Allosamedin reduced the level of esophageal remodeling as assessed by trichrome staining (**Fig 7**)($p <0.05$).





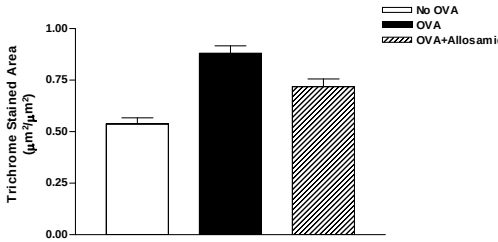


Fig 5: AAmcase activity

Fig 6: Eosinophils

Fig 7: Trichrome stain

Reportable Outcomes:

- Smad-3 deficient mice have significantly less esophageal fibrosis in a mouse model of egg induced EE.
- Targeting chitinase with allosamidin reduced eosinophilic inflammation in a mouse model of egg induced EE.
- Targeting chitinase with allosamidin reduced esophageal fibrosis in a mouse model of egg induced EE.

Conclusion:

Targeting Smad3 or chitinase both partially reduced esophageal fibrosis. The mechanism by which targeting chitinase or Smad 3 reduces remodeling occurs at different steps in the eosinophil/TGF-b/Smad3 pathway as allosamidin inhibits both eosinophilic inflammation and fibrosis, whereas targeting smad3 only reduces fibrosis and does not effect numbers of eosinophils or TGFb+ cells. The combination of targeting Smad3 and chitinase may be more effective than targeting each pathway alone.

References:

None.

Appendices:

None.

Manuscripts/Reprints, Abstracts:

We plan to prepare a manuscript describing our findings once the remaining analyses are completed in year 3.